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Chapter 10

COVER CROPS, TILLAGE, AND GLYPHOSATE EFFECTS ON CHEMICAL AND BIOLOGICAL PROPERTIES OF A LOWER MISSISSIPPI DELTA SOIL AND SOYBEAN YIELD

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ABSTRACT

The adoption of sustainable cropping systems, including cover crops and no-tillage practices can promote soil conservation and improve soil quality. However, the selection of the best management practices to increase crop production is needed. A field study was conducted from 2001 to 2005 at Stoneville, MS, on a Dundee silt loam soil to assess the effects of cover crop (rye [*Secale cereale* L.], hairy vetch [*Vicia villosa* Roth], or none), tillage [conventional tillage (CT) or no-tillage (NT)] and herbicide (glyphosate or non-glyphosate post emergence), on soil chemical properties, soil microbial ecology, and soybean yield. Cover crops were killed before soybean (Glycine max L. Merr.) planting, incorporated into CT soils, and left on surface in NT soils. Soil (0 to 5 cm depth) was sampled at planting, and mid season following soybean planting. Soil was analyzed for total organic carbon (TOC), total nitrogen content (TNC), nitrate, electrical conductivity, and soil moisture. Biological parameters included fluorescein diacetate (FDA) hydrolysis, total bacteria, gram-negative bacteria and total fungi propagules, and microbial community analysis based on total fatty acid methyl ester (FAME) analysis. The greatest accumulation of TOC and TNC was under NT, particularly under cover crop management. NT soils, especially under cover crop management maintained the highest soil moisture content. Soils managed under a hairy vetch cover crop maintained at least two-fold greater soil nitrate and electrical conductivity compared to no cover crop regardless of tillage. FDA hydrolysis was 55 to 120% greater under NT compared to CT with the highest activity associated with cover crop managed soils. Patterns of microbial community structure were dependent on year and sample time dependent with a greater effect of tillage compared to cover crop. Soybean yields were consistently similar under CT and NT systems. Despite the beneficial effects on soil properties, soybeans grown with rye cover crop, regardless of CT or NT system, consistently yielded lower compared to hairy vetch or no cover crop. Adoption of cover crop-based systems by soybean farmers is less likely due to additional costs. Alternatively, soybean farmers are more likely to adopt NT-based production systems to potentially increase soybean yield and improve soil quality.

INTRODUCTION

One approach to achieve ecologically balanced crop production systems is the integration of cover crops and reduced tillage into various cropping systems (Magdoff, 2007). Winter cover crop can be used to suppress and/or replace unmanageable winter annual weed species and improve soil fertility and quality. The long growing season in the lower Mississippi Delta region permits the use of winter cover crops in row crop production (Reddy 2001, 2003; Reddy et al., 2003). Cover crops are planted in the fall and desiccated with herbicides the following spring before no-till planting of the summer crop (Reddy 2001, 2003, Reddy et al., 2003; Koger et al. 2005). Cover crops have long been used to reduce soil erosion and water runoff, improve water infiltration, soil moisture retention, soil tilth, organic carbon, and nitrogen (Mallory et al. 1998; Sainju and Singh 1997; Teasdale 1996; Varco et al. 1999; Yenish et al. 1996). Both rye and hairy vetch have been shown to enhance certain soil microbial populations in soybean and cotton (*Gosypium hirsutum* L.) production systems (Wagner et al., 1995; Zablotowicz et al., 1998). The effects of reduced and/or no tillage and crop rotations on altering soil microbial community structure has been shown in several geographical areas (Drijber et al, 1990; Feng et al., 2001; Lupwyai et al., 1993). Rye is often used as a cover crop because of its winter hardiness, abundant biomass production, and allelopathic nature that suppresses weeds by both physical and chemical interference (Barnes and Putnam 1986; Creamer et al. 1996). In addition to benefits provided by cereal cover crops, the legume cover crops biologically fix atmospheric nitrogen that subsequently becomes available during residue decomposition (Sainju and Singh 1997; Varco et al. 1999). Although natural winter vegetation provides soil cover in certain fields, it may not provide sufficient plant residues for longer periods like cover crop do (Reddy 2001, 2003).

The adoption of glyphosate-resistant cropping systems by North American farmers has greatly facilitated the use of reduced tillage practices (Cerderia and Duke, 2006; Locke et al., 2008). Although there appears to be a synergy between the use of glyphosate-resistant cropping systems and conservation practices, the interaction among these practices on soil quality has been assessed in few long-term field studies (Locke et al., 2008). This chapter will summarize the results of a study conducted to assess the contributions of various components of conservation systems: tillage, cover crops, and glyphosate use on soil properties, soil microbial ecology, and soybean yield. Soil microbial populations were assessed using cultural methods (plate counts) and community structure was determined using fatty acid methyl ester analysis (Cavigelli et al., 1995). In this chapter, we present results from the second phase of the study (2001-2005) that was initiated in 1997. The results of the first phase of the study (1997-2001) were published previously (Reddy et al., 2003).

MATERIALS AND METHODS

Experiment Description

Field studies were conducted from 2001 through 2005 at the USDA-ARS Southern Weed Science Research Farm, Stoneville, Mississippi (33°26′ N, 90°55′ W) in experimental plots as described earlier (Reddy et al., 2003). The soil was a Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqualf) with soil textural fractions of 26% sand, 56% silt, and 18% clay. Several properties of soils in these plots have been published previously (Reddy et al., 2003). The cover crop systems consisted of rye, hairy vetch, and no-cover crop. The no cover and rye cover crop plots were continuation of the same plots of a previous study (1997-2001), whereas, hairy vetch plots were previously under crimson clover cover crop system (Reddy et al., 2003). Rye and hairy vetch were drilled in 19-cm-wide rows using a no-till grain drill in mid-October of 2001, 2002, 2003, and 2004. Seeding rates were 80 kg ha^{-1} for rye and 30 kg ha^{-1} for hairy vetch. The entire experimental area was desiccated with paraquat at 1.1 kg ai ha^{-1} in mid-April of 2002, 2003, 2004, and 2005. At desiccation, all cover crops were in flowering stage. Hairy vetch was 25- to 40- cm tall and rye was about 100- to 125- cm tall. The two tillage systems were conventional tillage (CT) and no tillage (NT). The NT plots received no seedbed preparation tillage operations after the fall of 1997. After cover crop desiccation, the CT plots were tilled with a disk harrow and a field cultivator as needed to thoroughly incorporate the plant residue before soybean planting. In NT plots, the plant residue on the soil surface was left undisturbed. The CT plots were not tilled between soybean harvest and planting of cover crops in the fall.

Due to tillage operations required to prepare seedbed the CT plots, soybean was not planted immediately after desiccation of cover crops. At soybean planting, newly emerged vegetation after cover crop desiccation in all NT plots was controlled with paraquat at 1.1 kg ai ha^{-1} to facilitate soybean stand establishment. Glyphosate-resistant soybean cultivar 'AG 4702RR' was planted on 2 May 2002, 30 April 2003, and 3 May 2004, and 'AG4603RR' was planted on 18 April 2005. The two herbicide treatments were glyphosate- and non-glyphosate conventional herbicides applied post-emergence. In the glyphosate-based treatment, two applications of glyphosate at 0.84 kg ae ha^{-1} were applied. In the non-glyphosate-based treatment, acifluorfen at 0.28 kg ai ha^{-1} plus bentazon at 0.56 kg ai ha^{-1}, chlorimuron at 13 g ai ha^{-1} and clethodim at 0.14 kg ai ha^{-1} or fluazifop-P at 0.28 kg ai ha^{-1} were applied. Postemergence herbicides were applied between 3 to 8 weeks after soybean planting. Herbicide treatments were applied with a tractor-mounted sprayer with 8004 standard flatfan spray tips delivering 187 L ha^{-1} water at 179 kPa. No preemergence herbicides were used in the study. The experimental plots were not irrigated.

The experimental design was a split-split plot arrangement with four replications per treatment arranged in a randomized complete block design. Tillage (CT and NT) was the main plot, cover crops (rye, hairy vetch, and no-cover crop) was the subplot, and herbicide programs (glyphosate- and conventional herbicide) was the sub-subplot with four replications. Each sub-subplot consisted of 4 rows spaced 102 cm apart and was 24.3 m long. The same treatment was assigned to the same plot every year. Soybean was combine harvested from each plot, and grain yield was adjusted to 13% moisture.

Cover Crop / Spring Vegetation Biomass

Plant biomass was estimated from hairy vetch, rye, and no cover plots just prior to desiccation in 2002, 2003, and 2004. In 2005, plant residues were not determind as hairy vetch failed to establish. Plant residue was clipped at the soil surface from 1 m2 quadrants, oven-dried, and weighed. Because cover crop establishment was uniform throughout the experimental area, biomass was estimated only from for randomly selected spots considered as four replications.

Soil Sampling

Two soil samples were taken each year, the first prior to planting (April), and the second 14 days after the second post emergence herbicide application (midseason 42 to 46 days after planting). Each soil sample consisted of a composite of nine sub samples of 0- to 5-cm depth taken randomly from the middle two rows of each plot. To minimize the rhizosphere effect, soil was sampled in between the rows in the furrow. To avoid contamination, soil sampling probes were disinfected with isopropanol between plots.

Soil Chemical Analysis

Chemical analysis was conducted on air-dried soil that was passed through a 2-mm sieve and uniformly milled in a Wiley mill. Electrical conductivity (EC) and pH was determined in an aqueous soil suspension (2:1). Total organic carbon (TOC) and Total Nitrogen Content (TN) was determined on duplicate samples using a Flash EA 1112 elemental analyzer (CE Elantech, Lakewood, NJ). Water extractable anions were determined using ion chromatography (Dionex ICS 2000, Dionex Corp., Sunnyvale, CA). An IonPac AS18 hydroxide selective anion exchange column was used for anion separation (nitrate) with data analysis using Chromeleon software (Dionex Corp., Sunnyvale, CA).

Soil Enzymatic Activity

Soil hydrolytic activity was evaluated as an indicator of microbial activity, using fluorescein diacetate (FDA) as substrate modified from Schnürer and Rosswall (1982). Details of the method were reported elsewhere (Zablotowicz et al., 1998, 2000a, 2000b).

Microbial Propagule Density

Estimates of culturable soil microbial propagules were determined in soil samples from all sampling dates by serial dilutions and spiral plating (Spiral Plater, Spiral Systems Instruments, Bethesda, MD) on selective and semi-selective growth media, as described previously (Zablotowicz et al. 1998; Reddy et al., 2003; Zablotowicz et al 2007). Total heterotrophic bacteria, and gram-negative bacteria, were enumerated on 10% tryptic soy agar containing 100 μg cycloheximide ml^{-1}, 10% and tryptic soy agar with crystal violet (5μg ml^{-1}) and cycloheximide, respectively. Total fungal populations were determined on rose bengal-ammended potato dextrose agar (Martin 1950). Colonies of gram-negative bacteria, total fungi, and total bacteria were counted after 3, 5, and 7 d of incubation at 28 °C, respectively. Microbial propagule densities were expressed as $\log_{10}$ colony forming units g^{-1} soil (oven-dry wt).

Microbial Community Structure Characterization by Fatty Acid Methyl Ester Analysis

A protocol for Fatty Acid Methyl Ester (FAME) analysis was modified from that of Schutter and Dick (2000) and is described in detail elsewhere (Weaver et al., 2007; Zablotowicz et al., 2007a; Locke et al., 2008). After methylation and extraction, FAMEs were separated, identified, and quantified using an Agilent 6890 gas chromatograph and the MIDI EUKARYOTE protocol and verified by using MIDI FAME standards (Microbial ID, Newark, NJ).

Statistical Analysis

Data on soil properties were subjected to analysis of variance using PROC Mixed to assess the effects of tillage (NT or CT), cover crop (none, rye, or hairy vetch) and herbicide (glyphosate or conventional herbicide regime) and the interaction of these variables (SAS 2001). Each sample for the four years of the study was analyzed separately. Data on spring vegetative biomass at time of planting, were subjected to analysis of variance using PROC GLM (SAS 2001). Treatments means were separated at the 5% level of significance using Fisher's protected LSD test.

Differences and changes in microbial community structure were detected and described using principal component analysis (SAS, PROC PRINCOMP). Fatty acids that were very rare were excluded from analysis to reduce minor experimental variation (Zablotowicz et al., 2007; Locke et al., 2008). Following principal component analysis, the contributions of cover crop, tillage, herbicide, and interactions between cover crop and tillage on principal components were analyzed using SAS PROC MIXED. Pearson's correlations were conducted using SAS PROC CORR to determine major fatty acids contributing to the principal components, as well as to determine soil biological and chemical properties contributing to the principal components.

RESULTS AND DISCUSSION

Spring Vegetative Biomass

No winter weed control was used and plots not seeded in cover crops produced abundant biomass (1668 to 2926 kg dry matter ha^{-1}) from native winter and spring vegetation (Table 1). By comparison the highest plant residue was produced in rye plots (3143 to 4524 kg dry matter ha^{-1}).

Table 1. Cover crop dry biomass remaining on the surface of soil at soybean planting in no tillage plots in 2002 to 2004, Stoneville, Mississippi

Cover crop	2002	2003	2004
	Dry biomass (kg ha^{-1})		
No cover crop	2926 b*	2209 b	1668 b
Hairy vetch	3375 b	2973 a	2190 b
Rye	4524 a	3143 a	3280 a

*Mean of four replications. Means followed by the same letter do not differ at the 95% confidence level. Dry biomass in no cover crop was from winter annuals.

Plant residues in hairy vetch plots were intermediate (2190 to 3375 kg dry matter ha^{-1}), and were higher than no cover plots in 2003. Although plots were seeded twice, there was no establishment of hairy vetch in 2005, because of damping-off. Subsequently plant residue mass was not determined in 2005. Leguminous crops were sown into the hairy vetch plots for eight consecutive years, and high disease pressure from seedling pathogens (*Pythium*, *Rhizocotonia*, *Fusarium* and *Thielaviopsis*) may have developed in these soils. Other research from these experimental plots indicate that CFU of the fungus causing soybean charcoal rot *Macrophomina phaseolina* (Tassi) Gord, was greater under CT than NT soils and higher CFU was associated with a hairy vetch compared to rye or no cover crop (Mengistu et al., In Press). Other researchers found that use of a hairy vetch cover crop resulted in significant reduction of other soil-born fungi including *Rhizoctonia solani* and *Thielaviopsis basicola* (Delgado et al., 2006; Rothrock and Kirkpatrick, 1995).

Soil Moisture

This study was conducted under non-irrigated conditions and soil moisture can be a limiting factor affecting soybean production in the lower Mississippi Delta region. Thus best management practices that conserved moisture have the potential to increase soybean yield. For all four years (2002-2005), soil moisture at planting or mid season was significantly higher in NT compared to CT plots, regardless of cover crop or glyphosate use (Table 2). Similarly, with the exception of 2002 soil moisture was at least 9% higher in rye cover plots compared to no cover crop at planting. Conversely, soil moisture in hairy vetch plots was only higher than no cover in 2002 and 2004 at planting. The conservation of soil moisture under cover crops was short lived as the only effect on mid season moisture content was for rye in 2005.

Table 2. Effect of tillage and cover crops on soil (0 to 5 cm depth) moisture content determined at planting and mid season (seven weeks after planting), 2002 to 2005, Stoneville, Mississippi

Sample	Treatment	2002	2003	2004	2005
		Moisture content (g kg^{-1})			
At planting	Conventional tillage	151	159	200	172
	No-tillage	242	216	271	242
	LSD 0.01 [a]	7	3	30	10
Mid season	Conventional tillage	47	136	123	84
	No-tillage	69	167	193	105
	LSD 0.01	18	22	40	17
At planting	No cover	167	178	216	191
	Rye	210	198	236	230
	Hairy Vetch	214	184	253	199
	LSD 0.01	40	NS[b]	23	14
Mid season	No cover	53	148	141	83
	Rye	59	164	162	104
	Hairy Vetch	62	143	172	95
	LSD 0.01	NS	NS	NS	12
		P values for the analysis of variance components for soil moisture			
At planting	Tillage (Til)	<0.001	<0.001	<0.001	<0.001
	Cover crop (CC)	0.013	0.113	0.005	0.009
	Til x CC	0.149	0.864	0.305	0.134
	Herbicide	0.159	0.744	0.156	0.649
Mid season	Til	0.004	0.003	<0.001	0.004
	CC	0.068	0.093	0.233	0.029
	Til x CC	0.014	0.577	0.334	0.029
	Herbicide	0.639	0.218	0.315	0.019

[a] Fisher's least significant difference [b] NS = not significant.

The benefits of rye cover crop mulch in retaining soil moisture have been demonstrated in studies by Liebl et al (1992). These data indicate that higher soil moisture levels in either NT or cover crops under non-irrigated systems in the Mississippi conditions. Soybean production under non-irrigated condition provides relatively low gross return with a narrow margin of profit in the lower Mississippi Delta region (Heatherly et al., 2003), consequently conservation of moisture under a NT management should aid in maintaining soybean productivity under non-irrigated conditions.

Soil Total Organic Carbon and Total Nitrogen Content

Pooled over all years, NT maintained 54 to 100% greater TOC compared to CT, regardless of cover crop or glyphosate use (Table 3). TOC was higher in soils from plots with either rye or hairy vetch regardless of tillage or glyphosate with minimal tillage*cover crop interaction. These trends are consistent with previous reports (Reddy et al., 2003. During the four years of the current study there was a consistent increase in TOC in CT plots due to the effect of organic matter addition from incorporation of the cover crops. There was no effect of four years of continuous herbicide regime on TOC accumulation as similar levels of TOC were observed under a continuous glyphosate regime as under conventional herbicide management.

Table 3. Effect of tillage and cover crops on soil (0 to 5 cm depth) total organic carbon determined at planting and midseason (seven weeks after planting), 2002 to 2005, Stoneville, Mississippi

Sample	Treatment	2002	2003	2004	2005
		Total organic carbon (g kg^{-1})			
At planting	Conventional tillage	9.9	10.9	10.3	11.2
	No-tillage	15.8	16.8	18.1	19.4
	LSD 0.01 [a]	0.9	3.0	2.0	1.8
Midseason	Conventional tillage	10.2	10.5	10.1	10.3
	No-tillage	16.6	16.8	19.5	20.7
	LSD 0.01	2.1	0.5	1.9	1.0
At planting	No cover	10.5	11.8	12.4	14.0
	Rye	13.8	15.0	14.6	15.5
	Hairy Vetch	14.1	14.7	15.5	16.4
	LSD 0.01	0.5	2.3	2.0	0.2
Midseason	No cover	11.2	11.5	13.5	12.9
	Rye	16.3	15.1	14.9	16.6
	Hairy Vetch	15.4	14.5	16.0	17.0
	LSD 0.01	2.6	0.8	2.3	2.3
		P values for the analysis of variance components for total organic carbon			
At planting	Tillage (Til)	0.007	<0.001	<0.001	<0.001
	Cover crop (CC)	<0.001	0.011	<0.001	0.045
	Til x CC	0.086	0.302	0.620	0.605
	Herbicide	0.334	0.287	0.368	0.772
Midseason	Til	<0.001	<0.001	<0.001	<0.001
	CC	0.007	<0.001	0.017	<0.001
	Til x CC	0.14	<0.008	0.553	0.1653
	Herbicide	0.358	0.395	0.327	0.534

[a] Fisher's least significant difference [b] NS = Not significant.

TNC was significantly (<0.0001) influenced by tillage and cover crop at planting and mid season in all years with a significant interaction of tillage and cover crop observed in 2005 at planting and in 2003 and 2005 at the midseason sampling (Table 4). TNC in soils at planting were 55 to 69% greater in NT compared to CT soils, while cover crop managed soil had 22 to 38% greater TNC compared to no cover soils. Hairy vetch would be expected to increase total nitrogen content in soil as it fixes atmospheric nitrogen. However, the TNC of hairy vetch soils was significantly different compared to rye soils only at planting and mid season in 2003 and 2004. The long-term benefits of no-till on improving soil carbon accumulation have been well documented as reviewed elsewhere (Reeves, 1997; Locke et al., 2002, 2003, 2008). These current studies indicate that cover crop management can effectively improve reserves of both organic carbon and nitrogen (McVay et al., 1989).

Table 4. Effect of tillage and cover crop on total nitrogen content in soils (0 to 5 cm depth) sampled at planting and midseason (seven weeks after planting), 2003-2005, Stoneville, Mississippi

Sample	Treatment	2003	2004	2005
		Total nitrogen content (g kg^{-1})		
At planting	Conventional tillage	0.76	1.13	1.34
	No-tillage	1.25	1.91	2.08
	LSD 0.01 [a]	0.20	0.18	0.10
Midseason	Conventional tillage	1.19	1.36	1.64
	No-tillage	1.91	2.16	2.45
	LSD 0.01	0.21	0.17	0.23
At planting	No cover	0.83	1.33	1.56
	Rye	1.07	1.55	1.78
	Hairy Vetch	1.11	1.71	1.77
	LSD 0.01	0.05	0.17	0.16
Midseason	No cover	1.08	1.64	1.83
	Rye	1.67	1.73	2.03
	Hairy Vetch	1.89	1.92	2.28
	LSD 0.01	0.16	0.21	0.23
	P values for the analysis of variance components for total nitrogen content			
At planting	Tillage (Til)	<0.001	<0.001	<0.001
	Cover crop (CC)	0.014	<0.001	<0.001
	Til x CC	0.626	0.870	0.001
	Herbicide	0.647	0.884	0.521
Midseason	Til	<0.001	<0.001	<0.001
	CC	<0.001	0.005	<0.001
	Til x CC	0.002	0.634	<0.001
	Herbicide	0.391	0.342	0.27

[a] Fisher's least significant difference.

Soil Nitrate and Electrical Conductivity

Soil nitrate levels in soil sampled at planting were significantly greater in NT compared to CT soils in 2004 and 2005, and greater in NT compared to CT at mid season only in 2005, regardless of cover crop and glyphosate use (Table 5). However, across all years, the highest levels of nitrate were observed in soil from hairy vetch plots, regardless of tillage or glyphosate. For example, hairy vetch typically maintained about two-fold greater soil nitrate levels compared their respective no cover plots under CT or NT regimes at both sample dates between 2002 and 2004.

Table 5. Effect of tillage and cover crops on soil (0 to 5 cm depth) nitrate concentration at planting and midseason (seven weeks after planting), 2002 to 2005, Stoneville, Mississippi

Sample	Treatment	2002	2003	2004	2005
		Nitrate (mg kg^{-1})			
At planting	Conventional tillage	48	30	39	23
	No-tillage	57	36	71	43
	LSD 0.01 [a]	NS[b]	NS	13	16
Midseason	Conventional tillage	117	30	91	96
	No-tillage	132	20	73	61
	LSD 0.01	NS	NS	NS	19
At planting	No cover	37	19	44	30
	Rye	39	19	38	25
	Hairy Vetch	83	61	85	45
	LSD 0.01	17	8	15	7
Midseason	No cover	78	20	63	67
	Rye	88	14	63	68
	Hairy Vetch	207	42	120	100
	LSD 0.01	46	18	18	24
		P values for the analysis of variance components for nitrate concentration			
At planting	Tillage (Til)	0.232	0.103	<0.001	0.006
	Cover crop (CC)	<0.001	<0.001	<0.001	<0.001
	Til x CC	0.456	0.420	0.655	0.114
	Herbicide	0.086	0.312	0.505	0.308
Midseason	Til	0.310	0.188	0.261	<0.001
	CC	<0.001	<0.001	<0.001	<0.001
	Til x CC	0.002	0.427	0.535	0.734
	Herbicide	0.940	0.481	0.296	0.140

[a] Fisher's least significant difference [b] NS = Not significant.

Generally soils from hairy vetch plots maintained about two-fold greater soil nitrate levels compared to their respective plots under CT or NT regimes at both sample dates between 2002 and 2004. In 2005 when hairy vetch was not established, nitrate concentrations in hairy vetch soils were about 50% greater than no cover crop soils. The contribution of hairy vetch cover crop to nitrogen requirement in corn has been well documented (Koger and Reddy 2005; Teasdale 1996; Fortuna et al. 2008). No consistent effect of tillage or cover crop was observed on other anions e.g., sulfate and phosphate (data not shown).

Electrical conductivity was significantly increased by NT compared to CT in three of the four years regardless of cover crop or glyphosate use (Table 6), as has been previously reported at this site (Reddy et al., 2003). However, cover crop, specifically hairy vetch, had the greatest effect on EC. Soils maintained under hairy vetch had from 49 to 109% greater EC compared to no cover crop, with the lowest response in 2005, when hairy vetch failed to establish.

Table 6. Effect of tillage and cover crops on soil (0 to 5 cm depth) electrical conductivity at planting and midseason (seven weeks after planting), 2002 to 2005, Stoneville, Mississippi

Sample	Treatment	2002	2003	2004	2005
		Electrical conductivity (µS cm^{-1})			
At planting	Conventional tillage	67	77	88	129
	No-tillage	99	109	116	172
	LSD 0.01 [a]	NS [b]	22	22	NS
Midseason	Conventional tillage	115	109	134	133
	No-tillage	131	71	111	110
	LSD 0.01	NS	11	14	17
At planting	No cover	55	64	110	110
	Rye	78	82	113	117
	Hairy Vetch	115	117	148	144
	LSD 0.01	26	26	17	22
Midseason	No cover	84	64	104	104
	Rye	111	82	113	116
	Hairy Vetch	174	117	148	144
	LSD 0.01	35	26	16	23
		P values for the analysis of variance components for total organic carbon			
At planting	Tillage (Til)	0.032	<0.001	0.001	0.018
	Cover crop (CC)	<0.001	<0.001	<0.001	<0.001
	Til x CC	0.525	0.765	0.947	0.011
	Herbicide	0.086	0.248	0.677	0.283
Midseason	Til	0.148	<0.001	<0.001	0.002
	CC	<0.001	<0.001	<0.001	<0.001
	Til x CC	0.289	0.844	0.361	0.223
	Herbicide	0.6147	0.429	0.018	0.013

[a] Fisher's least significant difference [b] NS = Not significant

By comparison rye plots had 20 to 44% greater EC compared to control plots in 2002 to 2004, but similar EC levels in 2005. Pooled across tillage and cover crops, plots receiving glyphosate had about 8% greater EC compared to non-glyphosate plots at mid season in 2004 and 2005. This may be partially explained by the liberation of organic acids from decaying weeds.

The increased EC observed under NT and a legume cover crop is consistent with that previously reported for these experimental plots (Reddy et al., 2003) when crimson clover was used instead of hairy vetch. Studies in a tropical Brazilian soil indicated no difference in EC in no-till compared to mouldboard plowing (Roldań et al., 2005). Electrical conductivity is a parameter being used to assess spatial variability of soil properties (Johnson et al., 2001) and define site-specific management practices for reduced chemical input systems such as efficient nitrogen use (Khosla et al. 2002). The increased nitrate levels in hairy vetch plots correlates with the highest EC values in these soils.

Fluorescein Diacetate Hydrolytic Activity

The hydrolysis of FDA was chosen as a model substrate to characterize total heterotrophic activity of the soil microbial community in response to crop management regimes as FDA is a generic substrate for a wide range of hydrolytic enzymes such as esterases, lipases and certain proteases (Zablotowicz et al., 2000b). In all years, mean FDA hydrolytic activity was 55 to 120% greater in NT compared to CT soil regardless of cover crop or glyphosate use (Table 7). Likewise in all years soil from cover crop plots had 28 to 58% greater FDA hydrolytic activity compared to no cover crop, regardless of tillage or glyphosate use. The effects of rye and hairy vetch on FDA hydrolytic activity were similar in all years at planting, while at mid season soil from rye plots had greater FDA activity compared to hairy vetch plots in 2003 and 2005. These results are in agreement with other studies (Wagner et al., 1995; Bandick and Dick, 1999; Mendes et al., 1999; Reddy et al., 2003; Zablotowicz et al; 1998, 2007b) where increased FDA activity has been associated with various cover crop management practices. However, studies by Gaston et al. (2003) found elevated FDA hydrolytic activity in a silt loam under NT compared to CT in Louisiana, while either a hairy vetch or wheat cover crop had no effect on FDA hydrolytic activity. The increased activity of FDA may be due to either increased populations of soil microflora, or elevated levels of available carbon sources that serve as substrates for the hydrolytic enzymes associated with NT or cover crop management.

Soil Microbial Populations

Estimates of culturable microorganisms (total fungi, and total and gram-negative bacteria) were enumerated during 2002-2004. Total bacteria CFU were similar at planting and mid season under NT and CT in 2002 and slightly higher under NT in 2003 and 2004 for both planting and midseason averaged across all cover crop and herbicide regimes (Table 8). The greatest enrichment of total bacterial CFU was found in relation to cover crop with a significant increase in total bacteria associated with cover crops in all samples except mid season 2002.

Table 7. Effect of tillage and cover crops on soil (0 to 5 cm depth) fluorescein diacetate hydrolytic activity at planting and midseason (seven weeks after planting), 2002 to 2005, Stoneville, Mississippi

Sample	Treatment	2002	2003	2004	2005
		Fluorescein formed (nmol g^{-1} h^{-1})			
At planting	Conventional tillage	98	104	143	79
	No-tillage	215	162	294	175
	LSD 0.01 [a]	80	36	22	19
Midseason	Conventional tillage	111	109	121	248
	No-tillage	204	164	234	330
	LSD 0.01	12	18	30	39
At planting	No cover	119	106	174	96
	Rye	164	159	224	133
	Hairy Vetch	187	140	258	152
	LSD 0.01	39	25	35	23
Midseason	No cover	125	122	148	248
	Rye	183	158	198	320
	Hairy Vetch	163	130	187	254
	LSD 0.01	53	22	37	39
		P values for the analysis of variance components for FDA-hydrolytic activity			
At planting	Tillage (Til)	0.0023	<0.0001	<0.0001	<0.001
	Cover crop (CC)	<0.0001	0.0001	<0.0001	<0.001
	Til x CC	0.0619	0.3722	0.6064	<0.001
	Herbicide	0.1207	0.7072	0.1076	0.1243
Midseason	Til	0.016	<0.001	<0.001	<0.001
	CC	0.016	<0.001	0.002	<0.001
	Til x CC	0.245	0.179	0.247	<0.001
	Herbicide	0.316	0.615	0.648	0.001

[a] Fisher's least significant difference

A significant tillage by cover crop interaction was observed in two of the six sampling dates, in that a greater enrichment of total bacteria due to cover crop was observed when rye was incorporated by tillage compared to no cover CT.

Tillage had a minor effect on gram-negative bacteria at planting (NT significantly greater than CT only in 2003) pooled across cover crop and herbicide regimes. In the mid season sample, there was a greater abundance of gram-negative bacteria CFU in NT compared to CT all three years pooled across cover crops and glyphosate use. These results are not as easy to interpret because a significant tillage by cover crop interaction was observed in all at planting samples and one mid season sample (2004). The effects of cover crop with significantly higher gram negative CFU in rye plots compared to no cover crop for all sample dates. Gram negative bacteria are typically higher under moist conditions and the higher CFU under rye cover crops may be due to both increased carbon substrate and moisture. Gram-negative bacteria especially *Pseudomonas* spp. are associated with plant growth promoting activity as well as antagonism to phytopathogenic bacteria and may contribute to soil health under cover crop management (Kloepper et al., 1989).

Table 8. Effect of tillage and cover crops on soil (0 to 5 cm depth) total bacterial colony forming units at planting and midseason (seven weeks after planting), 2002 to 2004 Stoneville, Mississippi

Sample	Treatment	2002	2003	2004
		Total bacteria (log (10) CFU·g^{-1})		
At planting	Conventional tillage	8.13	8.09	8.21
	No-tillage	8.11	8.15	8.33
	LSD 0.05 [a]	NS [b]	0.03	0.05
Midseason	Conventional tillage	7.90	7.68	7.78
	No-tillage	7.87	7.81	8.07
	LSD 0.01	NS	0.12	0.25
At planting	No cover	7.91	7.93	8.09
	Rye	8.13	8.20	8.34
	Hairy Vetch	8.31	8.24	8.46
	LSD 0.01	0.17	0.06	0.08
Midseason	No cover	7.85	7.69	7.61
	Rye	7.99	7.77	8.11
	Hairy Vetch	7.82	7.78	8.05
	LSD 0.01	NS	0.08	0.17
Planting	Non-glyphosate	7.92	7.74	8.30
	Glyphosate	7.92	7.76	8.29
	LSD 0.05	NS	NS	NS
Midseason	Non-glyphosate	7.92	7.74	8.04
	Glyphosate	7.92	7.76	7.81
	LSD 0.05	NS	NS	0.04
		P values for the analysis of variance components for total bacteria		
At planting	Tillage (Til)	0.5653	0.017	0.0401
	Cover crop (CC)	<0.0001	<0.0001	<0.0001
	Til x CC	0.0347	<0.0001	0.0512
	Herbicide	0.9108	0.5974	0.9421
Midseason	Til	0.683	<0.001	0.0392
	CC	0.887	0.003	<0.001
	Til x CC	0.707	0.093	0.004
	Herbicide	0.390	0.289	0.006

[a] Fisher's least significant difference [b] NS = Not significant.

Table 9. Effect of tillage and cover crops on soil (0 to 5 cm depth) gram-negative bacterial colony forming units, at planting and midseason (seven weeks after planting), 2002 to 2004, Stoneville, MS

Sample	Treatment	2002	2003	2004
		Gram negative bacteria (log (10) CFU g^{-1})		
At planting	Conventional tillage	6.80	6.46	6.37
	No-tillage	6.70	6.81	6.43
	LSD 0.01 [a]	NS [b]	0.30	NS
Midseason	Conventional tillage	5.43	5.83	6.37
	No-tillage	5.73	6.03	6.73
	LSD 0.05	0.22	0.16	0.11
At planting	No cover	5.53	6.68	5.18
	Rye	5.75	6.70	5.24
	Hairy Vetch	5.47	6.89	5.32
	LSD 0.01	0.20	0.12	0.12
Midseason	No cover	5.53	5.88	6.38
	Rye	5.75	6.02	6.62
	Hairy Vetch	5.47	5.89	6.58
	LSD 0.01	0.20	0.12	0.11
		P values for the analysis of variance components for gram-negative bacteria		
At planting	Tillage (Til)	0.169	0.002	0.153
	Cover crop (CC)	<0.001	0.001	<0.001
	Til x CC	<0.001	0.013	<0.001
	Herbicide	0.007	0.065	0.431
Midseason	Til	0.014	0.037	<0.001
	CC	0.028	0.047	<0.001
	Til x CC	0.225	0.112	0.001
	Herbicide	0.264	0.225	0.006

[a] Fisher's least significant difference [b] NS = Not significant.

Estimates of total soil fungal populations were consistently greater under NT compared to CT soils, regardless of cover crop or glyphosate use (Table 10). The greatest increase in soil fungi CFU was associated with cover crop with the highest populations associated with hairy vetch in 2002 and 2003 (P >0.01), while fungal CFU had similar densities under rye and hairy vetch in 2004. Similar responses of increased soil fungi have been observed in soybeans grown under hairy vetch, rye, or ryegrass cover crop management in other studies (Wagner et al. 1995; Zablotowicz et al., 1998; Reddy et al., 2003; Zablotowicz et al., 2007b). Studies by Reeleder et al. (2006) found no significant effect of either tillage or a rye cover crop on total soil fungal CFU. However, that study indicated that one pathogenic group of fungi, *Pythium* was greater under CT plots, especially under a rye cover crop. Specific antagonism or stimulation of beneficial and phytopathogenic fungi by cover crops (Rothrock and Hargrove, 1983) and their relationship to soil bacterial microflora is an area worthy of further investigation. The study by Reeleder et al. (2006) also evaluated fungal populations in soil sampled to 10 cm, while the previously cited studies observed greater increase in fungal populations when soil was sampled at a shallower depth (2 or 5 cm deep).

Table 10. Effect of tillage and cover crops on soil (0 to 5 cm depth) total fungal colony forming units at planting and midseason (seven weeks after planting), 2002 to 2004, Stoneville, Mississippi

Sample	Treatment	2002	2003	2004
At planting	Conventional tillage	5.57	5.36	5.50
	No-tillage	5.74	5.52	5.61
	LSD 0.01 [a]	0.12	0.09	0.05
Midseason	Conventional tillage	5.30	5.20	5.13
	No-tillage	5.63	5.43	5.44
	LSD 0.01	0.13	0.16	0.06
At planting	No cover	5.44	5.22	5.35
	Rye	5.59	5.48	5.61
	Hairy Vetch	5.94	5.61	5.71
	LSD 0.01	0.15	0.10	0.08
Midseason	No cover	5.47	5.22	5.18
	Rye	5.50	5.44	5.34
	Hairy Vetch	5.42	5.26	5.31
	LSD 0.01	NS[b]	0.15	0.06
		P values for the analysis of variance components for total fungi		
At planting	Non-glyphosate	5.68	5.45	5.57
	Glyphosate	5.64	5.43	5.55
		NS	NS	NS
Midseason	Non-glyphosate	5.53	5.40	5.36
	Glyphosate	5.40	5.26	5.25
	LSD 0.01	0.13	0.07	0.04
At planting	Tillage (Til)	0.044	0.011	<0.001
	Cover crop (CC)	<0.001	<0.001	<0.001
	Til x CC	<0.001	0.135	0.003
	Herbicide	0.121	0.326	0.432
Midseason	Til	0.017	0.003	<0.001
	CC	0.413	0.033	<0.001
	Til x CC	0.320	0.276	<0.002
	Herbicide	0.001	<0.001	<0.001

[a] Fisher's least significant difference [b] NS = Not significant.

At time of planting there was no significant effect of herbicide on any of the general groups of microorganisms studied. However, at mid season a significant effect of herbicide regime on soil fungi was observed in that soils under glyphosate management had lower fungal CFU compared to conventional herbicide programs in 2003 and 2004. Greater populations of total bacteria and gram-negative bacteria were observed under a non-glyphosate herbicide program compared to glyphosate only in 2004. More effective weed control was observed under the glyphosate system and a greater abundance of weeds in the conventional herbicide program may have stimulated soil fungi through rhizosphere enrichment. The lower fungal and or bacterial CFU associated with the glyphosate management program is in contrast to results conducted on a Brazilian soil (Arújo et al., 2003) where increased fungal and actinomycete propagules were associated with glyphosate under *in vitro* conditions.

Soil Microbial Community Structure

The soil microbial community structure based on total FAMEs, assessed using principal component analysis is summarized in Figure 1, 2, and Table 11.

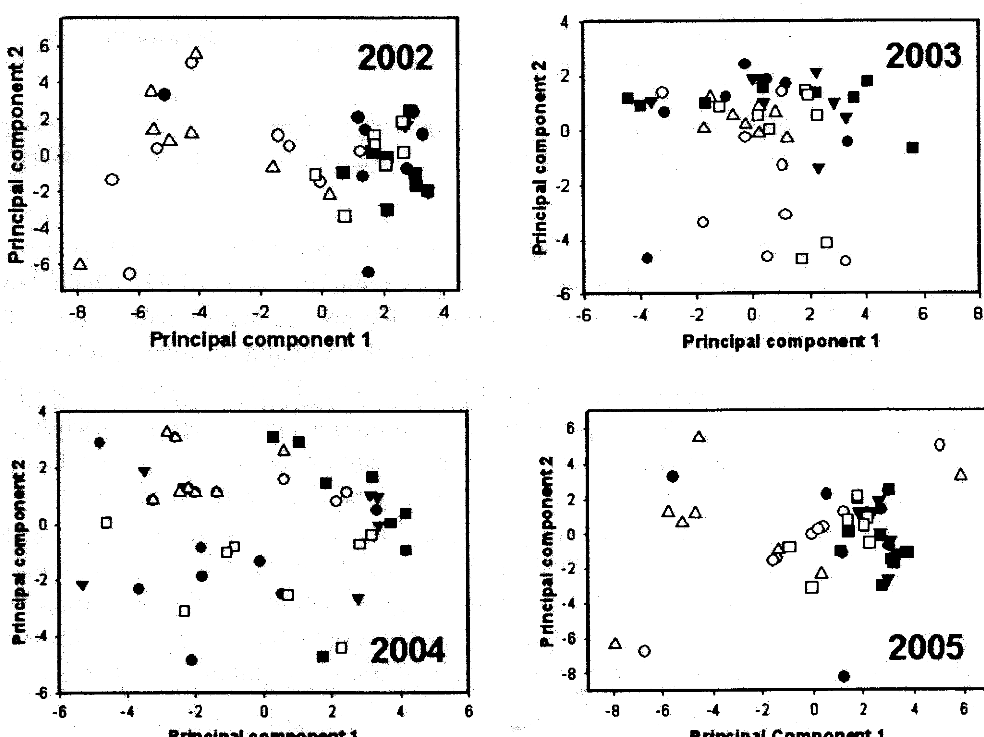

Figure 1. Principal component analysis of soil (0 to 5 cm depth) microbial community structure based on total fatty acid methyl esters, at planting 2002 to 2005, Stoneville, MS. Symbols for conventional till plots are unfilled and no-till plots are filled with no cover (●), rye (▼), and hairy vetch (■).

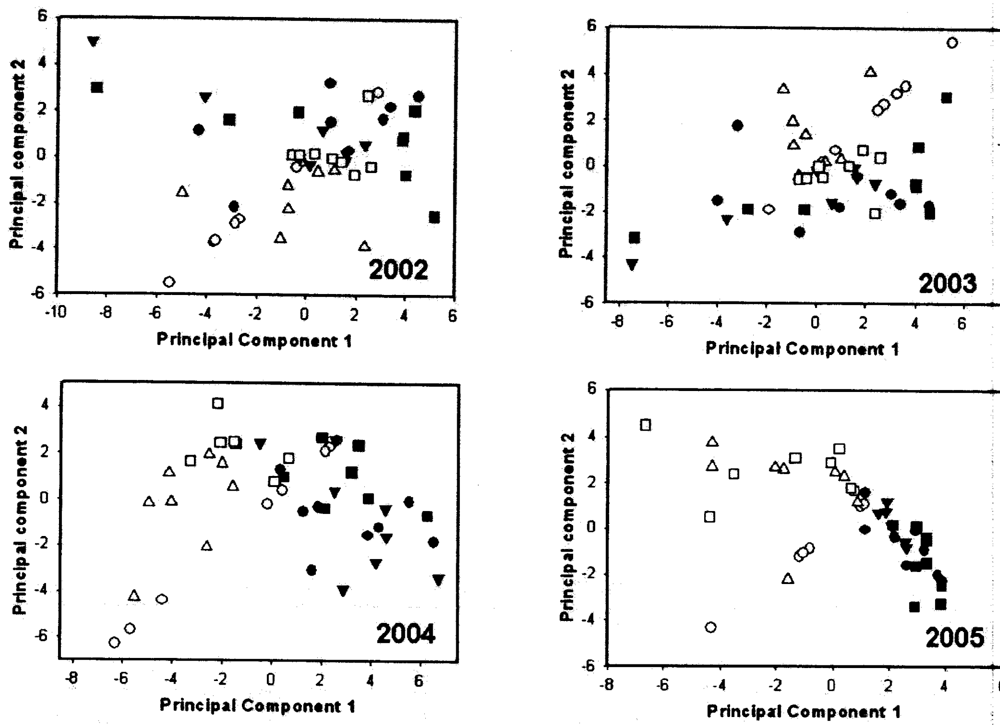

Figure 2. Principal component analysis of microbial community structure based on soil (0 to 5 cm depth) total fatty acid methyl esters, midseason (seven weeks after planting) 2002 to 2005, Stoneville, MS. Symbols for conventional till plots are unfilled and no-till plots are filled with no cover (●), rye (▼), and hairy vetch (■).

These results indicated a unique and dynamic microbial composition in each year of the study and even at sample time. For example, at planting in 2002, higher chain length unsaturated FAMEs 18:0 and 22:0, and the gram-positive branched fames 15:0iso and 17:0iso were among the factors in PC1, while in 2005 the low chain length saturated FAMES 15:0 and 14:0 and gram-negative hydroxylated FAME's 18:1wtOH, 17:1wtOH and 16:1 2OH had the greatest contribution to PC1. At planting, the microbial community structure was associated with crop management in that cover crop, tillage or the interaction of tillage and cover crop contributing to principal component 1 (PC1) in all four years (Table 11). Principal component 2 was less affected by management practices as cover crop only significantly contributed to principal component 2 (PC2) in 2003, and the interaction of cover crop and tillage contributed to PC2 only in 2004. Considering the correlation of soil properties with the two major principal components FDA hydrolytic activity and total fungi were the major biological properties correlating with PC1 or PC2 in 2003, while nitrate, EC, or SOM were major chemical properties correlating to PC1 or PC2 in 2003.

At the midseason sample tillage significantly contributed to PC1 in 2004 and 2005, and PC2 in 2002 and 2003. There was no significant contribution of cover crop to either PC1 or PC2 at mid season. At midseason, FDA hydrolysis was the dominant biological parameter correlated with microbial community structure, and TOC, TNC, EC, and nitrate were the chemical properties that had contributed to community structure.

Table 11. Analysis of variance of principal components of soil (0 to 5 cm depth) microbial community structure based on total fatty acid methyl esters, at planting and midseason (seven weeks after planting), 2002 to 2005, Stoneville, Mississippi

Principal component	Source	2002	2003	2004	2005
At planting					
PC1	Eigen value	32.2	34.3	31.8	32.6
	Tillage	0.045	0.367	0.202	<0.001
	Cover crop	<0.001	0.482	0.017	0.001
	Tillage * cover crop	<0.001	0.031	0.080	0.001
	Herbicide	0.408	0.477	0.097	0.041
PC2	Eigen value	18.5	19.8	21.0	
	Tillage	0.859	0.124	0.215	0.887
	Cover crop	0.779	0.019	0.352	0.885
	Tillage * cover crop	0.762	0.644	0.006	0.668
	Herbicide	0.990	0.485	0.934	0.973
Midseason					
PC1	Eigen value	42.6	43.6	36.3	39.8
	Tillage	0.528	0.419	<0.001	0.003
	Cover crop	0.248	0.192	0.268	0.161
	Tillage * cover crop	0.567	0.512	0.921	0.329
	Herbicide	0.577	0.512	0.921	0.309
PC2	Eigen value	18.3	18.4	14.7	15.0
	Tillage	0.032	0.042	0.535	0.075
	Cover crop	0.453	0.391	0.049	0.053
	Tillage * cover crop	0.091	0.054	0.050	0.091
	Herbicide	0.133	0.126	0.185	0.884

Studies by Schutter et al., 2001 and Schutter and Dick (2002) indicated that specific changes in microbial communities in response to cover crops changed over time and these changes were driven by total carbon and nitrogen and biological activities such as microbial biomass and respiration. Studies on the microbial community structure of a silt loam maintained under continuous NT or CT cotton production in Alabama (Feng et al., 2001) indicated that the greatest effects of reduced tillage are observed prior to planting. During the fallow period prior to planting, the bacterial community has a greater role in contributing to the total microbial community. However, later in the growing season environmental conditions associated with higher temperature and crop competition for moisture have a greater effect on the soil microbial community.

In contrast to this study, FAME-based microbial community structure was assessed in a cotton-corn rotation or monoculture managed under conventional or glyphosate resistant cropping system (Locke et al., 2008). Following five years of glyphosate management, the greatest differences in microbial communities were associated with soils planted with glyphosate-resistant crops compared to conventional corn and cotton, while the crop species had less of an effect. Other studies evaluating the short-term effects of glyphosate on soil microbial community structure on a similar Dundee silt loam found no effect of glyphosate application also using total FAME analysis (Weaver et al., 2007).

Soybean Yield

A summary of the effects of tillage, cover crops and glyphosate on soybean yield is presented in Table 12. Tillage had no effect on soybean yield with cover crops and herbicide having the greatest effect on yield. In all four years of the study rye significantly reduced yield compared to no cover crop by 15 to 33%. Similar yields were attained under hairy vetch cover crop compared to no cover crop. Although hairy vetch can provide additional nutritional stimulation associated with an increase in the nitrate mineralized from hairy vetch residue, this had little effect on yield potential of soybean.

Table 12. Pearson correlations of soil biological and chemical properties contributing to principal components of microbial community structure based on total fatty acid methyl esters, at planting and midseason (seven weeks after planting), 2002 to 2005, Stoneville, Mississippi

Principal Component	2002	2003	2004	2005
At planting				
PC1	FDA* <0.0001	None	FDA 0.0232	FDA 0.0005
	Fungi <0.0001		Fungi 0.033	Nitrate < 0.0001
	TOC <0.0001		Nitrate 0.007	TOC < 0.0001
	Nitrate < 0.0001		pH 0.0127	Moisture 0.009
	EC < 0.0001			
PC2	None	Fungi 0.018	None	None
		FDA 0.008		
		EC 0.004		
		TOC 0.018		
MidSeason				
PC1	TOC <0.001	None	FDA <0.001	FDA <0.001
			EC <0.001	TOC <0.001
			TOC < 0.001	TNC <0.001
			TNC <0.001	Nitrate 0.002
PC2	FDA 0.005	FDA 0.001	EC 0.002	None
	Moisture 0.001	TBac 0.034	Nitrate 0.002	
	EC 0.007	EC 0.001		
	TOC 0.013	TOC 0.007		

FDA = fluorescein diacetate hydrolytic activity; EC = electrical conductivity, TOC = total organic carbon, TNC total nitrogen content, TBAC = total bacteria.

Table 13. Effect of tillage, cover crop, and glyphosate on soybean yield in 2002 to 2005, Stoneville, Mississippi

Treatment	2002	2003	2004	2005
	Soybean yield (kg ha^{-1})			
Tillage				
Conventional tillage	2537	3392	2415	2707
No-tillage	2237	3308	2146	2778
LSD 0.05[a]	NS[b]	NS	NS	NS
Cover crop				
No cover	2623	3492	2365	3024
Rye	1774	2981	1983	2100
Hairy Vetch	2765	3575	2493	3103
LSD 0.05	308	154	196	201
Herbicide				
Non-glyphosate	2262	3191	2207	2430
Glyphosate	2513	3508	2354	3055
LSD 0.05	214	125	90	164
Tillage (T)	0.158	0.630	0.168	0.385
Cover crop (CC)	<0.001	<0.001	0.001	<0.001
T * CC	0.403	0.035	0.581	0.002
Herbicide (H)	0.024	<0.001	0.003	<0.001
T * H	0.725	0.546	0.002	<0.001
CC * H	0.658	0.738	0.456	0.358
T * CC * H	0.642	0.359	0.431	0.739

No preemergence herbicides were used. Glyphosate-based treatment received two postemergence applications of glyphosate. Non-glyphosate treatment received postemergence applications of acifluorfen, bentazon, chlorimuron, and clethodim or fluazifop-P.
[a] Fisher's least significant difference [b] NS = Not significant.

The consistent increase in soybean productivity was associated with the use of a glyphosate herbicide regime as glyphosate-managed soybeans yielded 6.6 to 25.7% greater compared to soybean managed under a conventional herbicide regime. Several factors may be associated with a loss of soybean productivity under the rye cover crop system. The resilience of rye straw can interfere with stand establishment and slow early soybean development. Secondly, rye produces a composite of allelopathic compounds (Barnes and Putnam, 1986). These allelopathic compounds may directly restrict soybean growth or indirectly by affecting the microbial community in a manner to affect pathogenic or growth promoting microorganisms. Previous studies on these plots (Reddy et al., 2003) indicated that rye had no effect on soybean yield, while the legume crimson clover significantly reduced yields although there was a significant herbicide by cover crop interactions affecting soybean yield.

The use of cover crops may affect the symbiotic relations of soybean with *Bradyrhizobium japonicum*, as nitrogen released from the degradation of hairy vetch residues may inhibit nitrogen fixation. Symbiotic characteristics, nodulation and acetylene reduction activity (ARA) were determined in 2004. Early nodulation was greatest under NT plots or CT and NT plots under rye cover crop management, with the lowest nodulation observed in soybean from hairy vetch plots (data not shown). Nitrogen fixation as estimated by ARA was initially the highest in NT plots without cover crops during the initial two samples. These results suggest that a delay in establishment of nitrogen fixation may be associated with soybean grown under soils previously cultivated to a hairy vetch cover crop. Nodulation is sensitive to available soil nitrogen and the high nitrate availability could inhibit initial establishment of the symbiosis (Streeter, 1988.).

Conclusions

Tillage and cover crops show beneficial effects on several parameters of soil quality. The consistently reduced soybean yield under rye cover crop illustrates a negative effect on soybean productivity, thus a rye cover crop is not recommended for soybean production in Mississippi. Hairy vetch had no deleterious effects on soybean productivity, however considering the costs associated with establishment of this cover crop, there is no economic benefit to the producer. Although soybean yield was not affected by NT practices, this management was facilitated by the use of a glyphosate based management system and does offer a clear economic benefit for the grower.

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